MONOZYGOUS TWINS DISCRIMINATION IN FORENSIC MEDICINE

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INTRODUCTION

Monozygotic twins are a challenge for forensics. Because of the fact that they originate from the same cellular mass, their genetic constitution is identical and that's why general molecular discrimination techniques can't be applied for identifying twins.

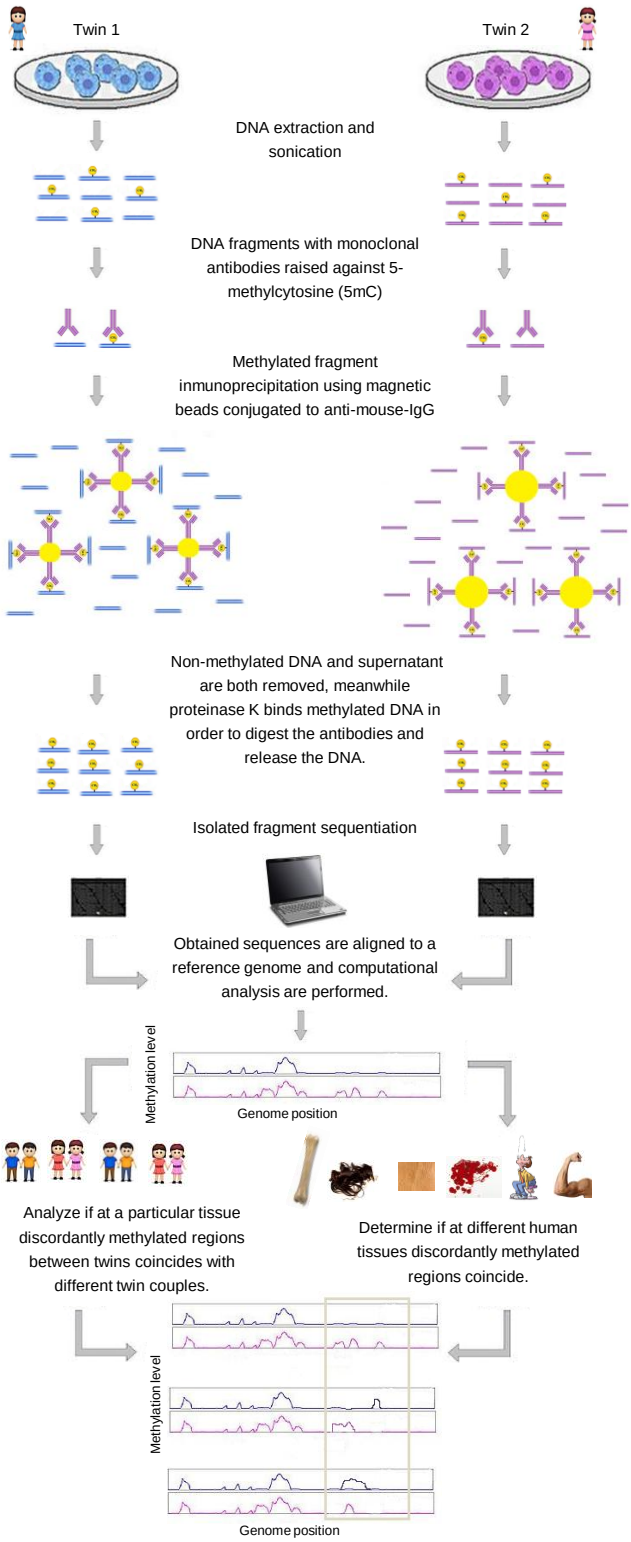
At genomic level, it can be seen that after the process of twinning, twin's genomes may diverge by accumulating somatic mutations. But this source of variation it's not useful for discriminate between identical twins at molecular level. Since genomic mutations are contingent and stochastic, they don't always take place at the same region between different twin pairs.

On the other hand, talking about epigenetics, clear evidences that justify the direct participation and the importance of epigenetics in shaping human phenotypic variability between twins are being found. It is believed that this might be a possible source for being able to discriminate twins as it has been seen that the genes which respond to external signaling tend to have different epigenetic patterns between twins.

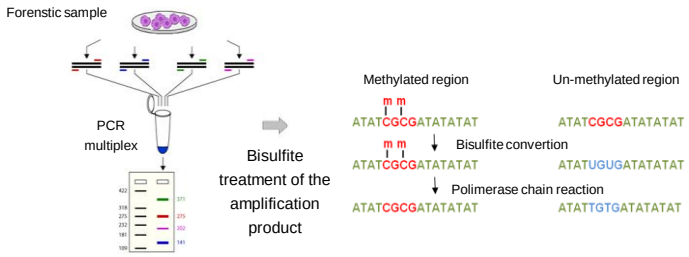
Therefore, it has been thought that different methylomas from different twins could be analyzed in order to see if specific regions might always suffer a different degree of metilation between twin pairs and different tissues. By doing this it could be possible to perform an automated forensic discrimination technique based on the analysis of these different methylated regions.

It was decided to study the methylome instead of the acetylation degree or histones methylation of forensics samples because when working with forensics samples, usually these are in a poor condition and the conservation of proteins or mRNA into the cell is terrible, whereas the DNA and its modifications have a better tolerance to adverse situations.

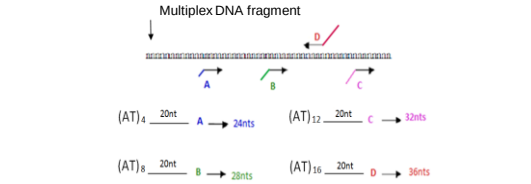
PROJECT FOR DISCRIMINATION MARKER DETECTION



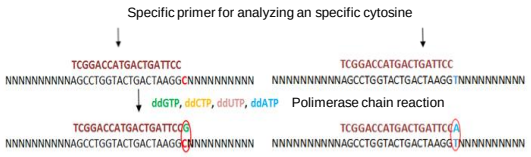
AUTOMATED TWIN DISCRIMINATION TECHNIQUE



PCR multiplex is performed with the different regions of the genome susceptible to have differences in methylation patterns between twins. This multiplex PCR uses primers that flank discordant regions with various cytosines methylated / unmethylated. Then, a bisulfite assay is carried out to the amplified DNA in order to discriminate methylated cytosines from non-methylated ones by creating SNPs C / T.

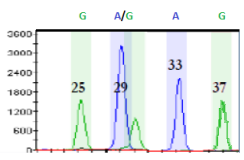


The methylation status of each cytosine can be detected by amplifying the SNP C / T using minisequentiation process which requires an specific primer design of those particular cytosine position.



The Taq extends each designed primer for each specific reagion with a single dideoxynucleotide that would send out different fluorescence depending on whether the SNP is a C or T. If there is a:

C → green emission will be seen.
T → blue emission will be seen.



To explain the minisenquentiation results an electrophoresis is performed. In the electropherogram, the X axis represents the primers size. In this way it is possible to identify which genome cytosine belongs to the fluorescent emission that is observed in each peak. The peak color makes reference to the methylation state of the cytosine:

-Green/Green: Homozygous cytosine methylation.
-Green/Blue: Heterozygous cytosine methylation.
-Blue/Blue: Homozygous cytosine non-methylation.

CONCLUSIONS

If the proposed research project was able to detect differently methylated regions between twins, the proposed discrimination technique would generate information about their genome´s 50 cytosine methylation status.

The probability for two twins to coincide in the methylation pattern of those 50 cytosines would be close to zero, so different methylation patterns will be obtained for different twins in order to being able to discriminate between twins biological samples.

REFERENCES

Pictures that appear at this poster are an adaptation of the images that can be found at the following websites:
•PREMIER Biosoft: «Multiplex PCR» in Premier Biosoft [online] Web. 30 May 2013 <http://www.premierbiosoft.com/tech_notes/multiplex-pcr.html>
•Servicio Central de Apoyo a la Invesigación: «Manual de Genotipado» in Universidad de Córdoba [online] Web. 30 May 2013 <http://www.uco.es/servicios/scai/impresos/GEN/Manual%20de%20Genotipado.pdf >
•Wikipedia «Methylated DNA immunoprecipitation» in Wikipedia [online] Web. 30 May 2013 <http://en.wikipedia.org/wiki/Methylated_DNA_immunoprecipitation >